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Physical and chemical factors influencing the toxicity of inorganic salts to Monilia sitophila (Mont.) Sacc.

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(WITH TWO TEXT FIGURES)

The many researches of recent years on the relative value of various media for growing bacteria and fungi, and the almost equally extended studies of the toxicity of various mineral salts for these organisms have not so far led to any very careful analysis of the physiological relations of toxic salts as affected by the media in which they are tested. This is especially true of the commonly used organic constituents of culture media, such as the sugars, proteids, etc. The study of the toxic action of salts when mixed together in aqueous solutions as compared with their effects when used separately has led to the very fruitful conception of balanced solutions, which plays so large a role in modern studies of osmosis, penetrability, the making of media, etc. The chemical interrelations of the salts and organic constituents of ordinary media are doubtless of fundamental significance in determining their physiological effects. The chemical relation of a so-called toxic salt to the medium in which it is offered must be understood if the real nature of the toxic effect is to be correctly analyzed. The compounds formed when dilute solutions of salts are mixed with carbohydrates, proteids, etc., are as yet little understood. The study of the relative physiological effects of series of such combinations may indirectly throw light on the nature of the compounds themselves and indicate the conditions under which they

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are formed. Of still greater significance, however, is the bearing of such studies on the actual determination of the relative toxicity of different substances and the fundamental questions as to the nature of toxic effects in general.

In a former paper (15) I have already shown that the toxic action of various nitrates on *Monilia sitophila* (Mont.) Sacc. is greatly influenced by the medium in which the fungus is grown at the time it is being poisoned. For example, ferric nitrate and aluminum nitrate were found to be much less toxic in peptone media than in glucose, fructose, galactose or starch media. Barium nitrate, on the other hand, was more toxic in peptone media than in glucose, fructose or starch. Through further experiments which it is the purpose of this paper to report, I have studied the influence of carbohydrates and peptone on the toxicity of eleven different chlorides. Observations on the germination and growth of *Monilia* in slightly toxic media has led to the suggestion that an important effect of a toxic substance may be to diminish the rate of water absorption by the fungus. In the hope that this suggestion might throw light on the conditions that obtain in poisoned cultures, I have also studied the influence of the water supply on the rate and amount of growth which *Monilia* makes when growing in various media.

As already noted the discovery of the mutual antagonism which exists between inorganic salts as regards their toxicity to plants has led to the conception of balanced solutions. Boehm (3) was perhaps the first to observe this relation. He found that the poisonous action of magnesium salts on bean plants could be counteracted by calcium carbonate. He also noted that calcium nitrate and calcium sulphate had an ameliorating influence on the toxicity of sodium and potassium salts. Von Raumer (26) also observed the great toxicity of magnesium salts in the absence of calcium. The literature on this subject has recently been summarized by McCool (21) That such mutual antagonism exists between a rather large number of salts is one of the best established facts of plant physiology.

In 1902 Loeb (18) attacked the further problem as to whether or not the presence of non-electrolytes would effect the toxicity of inorganic salts. Using the eggs of *Fundulus*, he measured the

toxicity of sodium chloride and zinc sulphate in the presence of small amounts of urea, alcohol, glycerine and cane sugar and concluded that these non-electrolytes do not influence the toxicity of either salt. The toxicity of zinc sulphate, however, was greatly reduced by cane sugar. This result Loeb attributed to the formation of zinc saccharate. Judging from what we know of the conditions under which saccharates are produced, we can hardly accept this explanation for the case of a dilute solution of zinc sulphate and cane sugar. Saccharates are obtained in strongly alkaline solutions. Dilute solutions of zinc sulphate are quite acid in reaction. I shall discuss this point further in connection with my own observations.

Lidforss (16) had long before observed that the toxicity of calcium nitrate, sodium nitrate and sodium chloride for germinating pollen grains was much reduced by the addition of ten per cent. of cane sugar to the solution. Wassermann and Takaki (33) found that an infusion made from cells of the central nervous system of the guinea-pig has an inhibiting action on the toxin of *Bacillus Tetani*. Like infusions made from kidney or liver cells did not counteract the poison. Loew (19) was not able to lessen the toxicity of magnesium salts for *Spirogyra* by the addition of methyl alcohol or glycerine to his solutions. Kahlenberg and True (13) claim that cane sugar has no effect on the toxicity of boric acid for *Lupinus albus*, but that the addition of dextrine to a mercuric chloride solution greatly reduces its toxicity. They were unable to precipitate mercuric oxide from such a solution by means of caustic alkali and assume that the mercury and dextrine combine to form a complex ion which is less toxic than the mercuric ion. Winogradsky and Omeliansky (36) found that peptone and glucose had an inhibiting action on the growth of the nitrifying bacteria. Duggar (6) obtained better germination of the uredospores of *Puccinia Helianthi* in distilled water than in one per cent. peptone solution. Stockard (31) reports that when cane sugar is added to a solution of lithium chloride or ammonium chloride the toxic action of these salts on *Fundulus* eggs seems to be augmented by the presence of the sugar, although the osmotic pressure of the sugar-salt mixture is considerably below that of sea-water.

Quite recently Ritter (28) has made a study of the effects of acids on Mucors growing in several different media. He finds that the toxicity of citric, malic, tartaric, hydrochloric and nitric acids is much reduced by the presence of peptone in the medium. When the source of nitrogen is ammonium citrate, ammonium tartrate, ammonium malate, asparagin or peptone, the toxicity of the various acids is much less than when the nitrogen is offered in the form of ammonium chloride, ammonium sulphate or ammonium nitrate. Grape sugar was found to reduce the toxicity of tartaric acid to a less extent than peptone.

A review of the literature shows that the influence of the more complex organic compounds on toxic substances has generally been neglected by those who have studied the effects of poisons on fungi. In my further studies on this point I have tested the influence of carbohydrates and peptone on the toxicity of a number of different chlorides, and have obtained evidence which suggests that an important factor in toxicity is the influence of the poison on the ability of the protoplast to absorb water. I have used as before the fungus *Monilia sitophila* in all of my experiments. Went (34) found that this fungus was able to use as a partial source of its food supply a rather large variety of organic compounds. This, together with the fact that it is a rapid grower, makes it especially well suited to such investigations. In experiments on the effects of toxic substances on fungi the choice of a standard by which to judge the degree of the poisonous action is a matter of some difficulty. One method is to grow the fungus in media containing the poison in different concentrations and then to determine the weight of the mycelium produced in the several media in a given period of time. On account of the difficulty in getting all of the mycelium out of the culture vessels and freeing it from constituents of the medium this method was not used. In all of my experiments, I have taken as the standard for judging toxicity, *the highest concentration of the poison in which any of the spores germinate after three days of incubation.* The spores are considered to have germinated if they show any visible evidence of growth when magnified to about one thousand diameters. I find this method to be both reliable and convenient.

My cultures were grown in pint milk bottles or in two hundred

and fifty cubic centimeter Jena flasks. New bottles and flasks were obtained and a different set was used for each of the salts tested. Thus the same flask was never used for testing more than one salt. This prevents errors which might arise through small traces of one salt being taken up by the glass and carried into cultures of some other salt. All glassware used in the making of media or the growing of cultures was first treated for at least twelve hours with a chromic acid cleaning solution. It was then thoroughly rinsed with tap water and drained. After draining for a short time it was again rinsed, first in distilled water and then in triple distilled water. The water used in the making of culture media was triple distilled, once from acid potassium dichromate, once from alkaline potassium permanganate and then again redistilled. This water gave a resistance of approximately one hundred and twenty thousand ohms. All of the salts used were Baker's analyzed chemicals, except in the case of barium chloride, cobaltous chloride and cadmium chloride, which came from Kahlbaum. The glucose, saccharose, soluble starch and peptone were obtained from Kahlbaum; the lactose came from Merck. Five per cent. solutions of each of these substances gave the following resistances; glucose, fifty-six thousand ohms; saccharose, forty-nine thousand ohms; lactose, twenty-seven thousand ohms; soluble starch, three thousand, three hundred ohms; and peptone two thousand ohms. A comparison of these resistances gives a good idea of the relative purity of the organic substances used.

The concentration of the salts in the various media are expressed in terms of molar solutions; these concentrations were made by dissolving some multiple or some fraction of a gram molecular weight in one liter of solution. Twenty cubic centimeters of medium were placed in each flask, and the flask and medium were sterilized for approximately ten minutes at 100° C. As soon as the medium had thoroughly cooled it was inoculated with fresh ripe spores of *Monilia*. The spores used in inoculating any given series of media were taken from the same culture. They were shaken from the culture flask on to a piece of white paper and were thoroughly mixed by means of a sterile scalpel. Approximately equal quantities of spores were dusted over the

surface of each medium. The cultures were then incubated in a room which was kept at a temperature of about 26° C. Observations were always made after an incubation period of three days. For the purpose of making careful microscopic observations, each culture was poured out into a small Petri dish, which could be placed on the stage of the microscope. All cultures were first observed under a magnification of about two hundred diameters. If this observation showed no germination, they were then more carefully studied under a magnification of approximately one thousand diameters.

In the tables that follow the plus sign indicates that the spores had germinated while the minus sign means that there was no germination in the medium in question. The results thus given in the tables refer always to the presence or absence of germinated spores in the different cultures, after an incubation period of three days. The minus sign does not mean that the spores will not germinate in the given medium but only that they have not germinated after three days. If left in the medium for a longer time the spores might germinate. *The highest concentration of a salt that will allow germination of the spores within three days is termed the limit concentration for that medium and period of time.* The toxicity of eleven different chlorides has been tested. Chlorides were selected because they have been much studied by other investigators and because they are, on the whole, more soluble than nitrates, sulphates or other common salts. In order to determine the influence of saccharose, glucose, lactose, starch and peptone on the toxicity of the several chlorides, I have used each of these substances separately in testing the toxicity of each inorganic salt. In distilled water and tap water the spores of *Monilia* germinate but produce only a very small amount of mycelium. Any of the organic substances used, when added to distilled water, greatly increase the growth, and they also tend to shorten the time required for germination. Abundant mycelium is produced in five per cent. solutions of glucose, saccharose, lactose, starch or peptone. When chlorides in sufficient concentration are added to any of these media the germination of the spores is completely inhibited. The approximate value of the limit concentration of the different salts in each medium was

obtained through a number of preliminary experiments. It was impossible to predict what the limit concentration would be in any case. My method was to make a guess at this value and then test the accuracy of the guess through experiments. In some cases only one preliminary experiment was necessary in order to determine the approximate value of the limit concentration of a given salt in one of the media. In other cases, however, several preliminary experiments had to be made before this value was ascertained. The data given in the tables was obtained in the final set of experiments and shows the great influence of the organic part of the medium on the toxicity of the different chlorides. The tables are arranged in the order of the toxicity of the salts tested. The influence of the medium on the toxicity of potassium chloride is shown in Table I.

TABLE I

THE TOXICITY OF POTASSIUM CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. KCl	Growth After 3 Days	G.M. Conc. KCl	Growth After 3 Days	G.M. Conc. KCl	Growth After 3 Days	G.M. Conc. KCl	Growth After 3 Days	G.M. Conc. KCl	Growth After 3 Days
2.3	−	2.3	−	1.6	−	1.5	−	1.5	−
2.2	−	2.2	−	1.5	−	1.4	−	1.4	−
2.1	−	2.1	−	1.4	−	1.3	−	1.3	−
2.0	−	2.0	−	1.3	−	1.2	−	1.2	−
1.9	−	1.9	−	1.2	−	1.1	−	1.1	+
1.8	+	1.8	−	1.1	+	1.0	−	1.0	+
1.7	+	1.7	+	1.0	+	.9	−	.9	+
1.6	+	1.6	+	.9	+	.8	+	.8	+
1.5	+	1.5	+	.8	+	.7	+	.7	+
1.4	+	1.4	+	.7	+	.6	+	.6	+

A glance at the table shows that the toxicity of potassium chloride is decidedly influenced by the medium in which it is offered. It is more than twice as toxic in starch as in saccharose or glucose. It is also more toxic in starch than in lactose and peptone. Its toxicity in lactose is the same as in peptone, but is about thirty-five per cent. less than in saccharose. It is most toxic in starch and least toxic in saccharose. The average of the limit concentrations in the five media is 1.1 molar and shows that potassium chloride is less toxic than any of the other chlorides that I have tested; this may be associated with the high nutrient

value of potassium. The toxicity of potassium chloride in starch media is the same as was found for potassium nitrate in the same medium.

TABLE II

THE TOXICITY OF AMMONIUM CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. NH_4Cl	Growth After 3 Days	G.M. Conc. NH_4Cl	Growth After 3 Days	G.M. Conc. NH_4Cl	Growth After 3 Days	G.M. Conc. NH_4Cl	Growth After 3 Days	G.M. Conc. NH_4Cl	Growth After 3 Days
.80	−	1.30	−	1.0	−	1.2	−	.95	−
.75	−	1.25	−	.9	−	1.1	−	.90	−
.70	+	1.20	−	.8	−	1.0	−	.85	−
.65	+	1.15	−	.7	−	.9	−	.80	−
.60	+	1.10	−	.6	+	.8	−	.75	−
.55	+	1.05	−	.5	+	.7	+	.70	+
.50	+	1.00	+	.4	+	.6	+	.60	+
.45	+	.90	+	.3	+	.5	+	.50	+
.40	+	.80	+	.2	+	.4	+	.40	+
.35	+	.70	+	.1	+	.3	+	.30	+

Table II shows that the toxicity of ammonium chloride is not greatly different in the various media, though some differences are to be noted. It is least toxic in glucose and most toxic in lactose. The toxicity of potassium chloride in starch is approximately the same as the toxicity of ammonium chloride in peptone, glucose and starch. The average limit concentration of ammonium chloride is .72 molar. When all of the media are taken into consideration, we see that ammonium chloride is more toxic than potassium chloride but less toxic than sodium chloride. Ammonium chloride is a possible source of nitrogen for *Monilia* and it may be that for this reason it is less toxic than sodium chloride.

The toxicity of sodium chloride in the different media as shown by Table III stands in sharp contrast with that of potassium chloride. While potassium chloride is least toxic in saccharose, sodium chloride is most toxic in this medium. It is hard to understand how two salts that are so much alike chemically should be so different as regards their toxicity in saccharose media. It is very interesting to see that potassium chloride, ammonium chloride and sodium chloride show almost equal toxicity in starch media, while in saccharose, glucose, lactose and peptone they

TABLE III

THE TOXICITY OF SODIUM CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. NaCl	Growth After 3 Days	G.M. Conc. NaCl	Growth After 3 Days	G.M. Conc. NACl	Growth After 3 Days	G.M. Conc. NaCl	Growth Afier 3 Days	G.M. Conc. NaCl	Growth After 3 Days
.56	−	1.30	−	.90	−	1.5	−	1.00	−
.40	−	1.20	−	.80	−	1.4	−	.95	−
.44	−	1.10	−	.70	−	1.3	−	.90	−
.38	−	1.00	−	.60	−	1.2	−	.85	−
.32	−	.90	−	.50	+	1.1	−	.80	−
.26	+	.80	+	.40	+	1.0	−	.75	+
.20	+	.70	+	.30	+	.9	−	.70	+
.14	+	.60	+	.20	+	.8	+	.65	+
.08	+	.56	+	.10	+	.7	+	.60	+
.02	+	.50	+	.05	+	.6	+	.55	+

differ widely. These differences may be due to the influence of the salts on the enzymes produced in the different media. Sodium chloride is least toxic in glucose, starch and peptone, the limit concentration being approximately the same in these three media. It is more toxic in saccharose than in lactose and more toxic in lactose than in glucose, starch or peptone. The average of the limit concentrations in the different media is .62 molar. When all of the media are thus taken into consideration we see that sodium chloride is much more toxic than potassium chloride.

TABLE IV

THE TOXICITY OF CALCIUM CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $CaCl_2$	Growth After 3 Days	G.M. Conc. $CaCl_2$	Growth After 3 Days	G.M. Conc. $CaCl_2$	Growth After 3 Days	G.M. Conc. $CaCl_2$	Growth After 3 Days	G.M. Conc. $CaCl_2$	Growth After 3 Days
.80	−	1.2	−	.60	−	1.1	−	.80	−
.70	−	1.1	−	.50	−	1.0	−	.70	−
.60	−	1.0	−	.40	−	.9	−	.60	−
.50	+	.9	−	.30	−	.8	−	.50	−
.40	+	.8	−	.20	−	.7	−	.40	+
.30	+	.7	+	.12	+	.6	−	.30	+
.20	+	.6	+	.11	+	.5	+	.20	+
.10	+	.5	+	.10	+	.4	+	.10	+
.05	+	.4	+	.09	+	.3	+	.09	+
.01	+	.3	+	.08	+	.2	+	.08	+

Calcium is one of the most important plant nutrients. It also has great value for counteracting the toxicity of other salts. The above table shows how its toxicity varies in the different media. Its poisonous action in starch is the same as in saccharose and only slightly less than in peptone. It is most toxic in lactose and least toxic in glucose. The average limit concentration is .44 molar.

TABLE V

THE TOXICITY OF BARIUM CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $BaCl_2$	Growth After 3 Days	G.M. Conc. $BaCl_2$	Growth After 3 Days	G.M. Conc. $BaCl_2$	Growth After 3 Days.	G.M. Conc. $BaCl_2$	Growth After 3 Days	G.M. Conc. $BaCl_2$	Growth After 3 Days
.70	−	.50	−	.55	−	.80	−	.50	−
.60	−	.40	−	.50	−	.70	−	.45	−
.50	−	.30	−	.45	−	.60	−	.40	−
.40	−	.20	+	.40	−	.50	−	.35	−
.30	−	.10	+	.35	+	.40	−	.30	−
.25	+	.09	+	.30	+	.30	−	.25	+
.20	+	.08	+	.25	+	.20	+	.20	+
.15	+	.07	+	.20	+	.10	+	.15	+
.10	+	.06	+	.15	+	.09	+	.10	+
.08	+	.05	+	.10	+	.08	+	.05	+

Table V gives the toxic values of barium chloride. Although barium is much like calcium chemically it is not a plant nutrient. Barium is on the whole much more toxic than calcium. It is less toxic in lactose media than in glucose. In this respect it is quite different from calcium chloride which is more than five times as toxic in lactose as in glucose. The average limit concentration is .26 molar. The toxicity of barium chloride is influenced less by the different media than that of any of the other chlorides which have been used. In saccharose, glucose, starch and peptone it is more toxic than calcium chloride. In lactose media, however, calcium chloride is more than three times as toxic as barium chloride. It is worth noting that the toxicity of the five chlorides as shown by the above tables is, on the average, as great in peptone as in the other media. Their toxicity is least in glucose and greatest in lactose. They are more toxic in peptone than in saccharose or glucose, although peptone is a much more favorable medium for the growth of the fungus. This shows that in some

cases at least, salts may be more toxic in a medium that is well suited to the growth of *Monilia* than in one which is less favorable.

TABLE VI

THE TOXICITY OF FERRIC CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $FeCl_3$	Growth After 3 Days	G M. Conc. $FeCl_3$	Growth After 3 Days	G.M. Conc. $FeCl_3$	Growth After 3 Days	G.M. Conc. $FeCl_3$	Growth After 3 Days	G.M. Conc. $FeCl_3$	Growth After 3 Days
.00050	−	.00050	−	.000400	−	.0013	−	.050	=
.00040	−	.00040	−	.000300	−	.0012	−	.045	−
.00030	−	.00030	−	.000200	−	.0011	−	.040	−
.00020	−	.00020	−	.000100	−	.0010	−	.035	+
.00010	+	.00010	+	.000080	−	.0009	−	.030	+
.00009	+	.00009	+	.000060	−	.0008	+	.025	+
.00008	+	.00008	+	.000040	−	.0007	+	.020	+
.00007	+	.00007	+	.000020	−	.0006	+	.015	+
.00006	+	.00006	+	.000010	+	.0005	+	.010	+
.00005	+	.00005	+	.000008	+	.0004	+	.005	+

Table VI shows that ferric chloride is far more toxic than the alkali and the alkali earth chlorides which have been tested. Although iron has a place among the nutrient elements it is nevertheless very poisonous. Of the alkali and alkali earth salts tested, potassium chloride is the least toxic. Iron chloride is least toxic of the salts of the heavy metals which have been used. It is very interesting to see that both potassium and iron are among the nutrient elements. This suggests that there may be some connection between toxicity and the nutrient relations of the elements. The effect of peptone on the poisonous action of ferric chloride is strikingly shown by the table. It is eight times more toxic in starch and ten times more toxic in lactose than in glucose and saccharose. In lactose it is more than three thousand times more toxic than in peptone. The average limit concentration in the different media is .007 molar.

Copper is known to be very poisonous to many algae and fungi, and is widely used in the making of fungicides. I find, however, that the chloride is, with the exception of iron, less toxic than any of the other chlorides of the heavy metals that have been tested. As shown by Table VII, cupric chloride, like ferric chloride, is more toxic in lactose than in saccharose, glucose, starch or peptone.

TABLE VII

THE TOXICITY OF CUPRIC CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $CuCl_2$	Growth After 3 Days	G.M. Conc. $CuCl_2$	Growth After 3 Days	G.M. Conc. $CuCl_2$	Growth After 3 Days	G.M. Conc. $CuCl_2$	Growth After 3 Days	G.M. Conc. $CuCl_2$	Growth After 3 Days
.00100	−	.00100	−	.000055	−	.00070	−	.050	−
.00095	−	.00095	−	.000050	−	.00065	−	.045	−
.00090	+	.00090	−	.000045	−	.00060	−	.040	−
.00085	+	.00085	−	.000040	−	.00055	−	.035	−
.00080	+	.00080	+	.000035	−	.00050	−	.030	−
.00075	+	.00075	+	.000030	+	.00045	+	.025	−
.00070	+	.00070	+	.000025	+	.00040	+	.020	+
.00065	+	.00065	+	.000020	+	.00035	+	.015	+
.00060	+	.00060	+	.000018	+	.00030	+	.010	+
.00055	+	.00055	+	.000015	+	.00025	+	.005	+

Its toxicity in glucose and saccharose is approximately the same. On the whole, cupric chloride is little more toxic than ferric chloride, its average limit concentration being .004 molar. The influence of the various media on the toxicity of copper is of special interest because the salts of this metal are so much used in the making of sprays. The action of lime on the copper in Bordeaux mixture is not thoroughly understood although this important subject has been given considerable attention (see Fairchild, **7**).

TABLE VIII

THE TOXICITY OF ZINC CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $ZnCl_2$	Growth After 3 Days	G.M. Conc. $ZnCl_2$	Growth After 3 Days	G.M. Conc. $ZnCl_2$	Growth After 3 Days	G.M. Conc. $ZnCl_2$	Growth After 3 Days	G.M. Conc. $ZnCl_2$	Growth After 3 Days
.00050	−	.000300	−	.00080	−	.000300	−	.050	−
.00045	−	.000250	−	.00075	−	.000200	−	.045	−
.00040	+	.000200	−	.00070	+	.000100	−	.040	−
.00035	+	.000150	−	.00065	+	.000080	−	.035	−
.00030	+	.000100	+	.00060	+	.000060	+	.030	−
.00025	+	.000050	+	.00055	+	.000040	+	.025	−
.00020	+	.000010	+	.00050	+	.000020	+	.020	−
.00015	+	.000008	+	.00045	+	.000010	+	.015	−
.00010	+	.000006	+	.00040	+	.000008	+	.010	+
.00005	+	.000004	+	.00035	+	.000006	+	.005	+

Table VIII shows the toxicity of zinc chloride. It will be seen that it is most toxic in starch and least toxic in peptone. The

most striking point in this table is the high toxicity of zinc in starch. In this medium it is more toxic than any of the other salts tested except mercuric chloride. This observation suggests that the value of zinc chloride for preserving wood from decay is based on a similar relation between zinc and the cellulose of the wood. Starch and cellulose are much alike chemically and it is rather to be expected that a salt which is extremely toxic in starch media would also be very toxic in cellulose. The toxicity of zinc chloride in saccharose and glucose is practically the same and is greater than in lactose. In lactose, zinc is more than fifty times less toxic than either copper or iron. The average limit concentration is .0022 molar.

TABLE IX

THE TOXICITY OF COBALTOUS CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $CoCl_2$	Growth After 3 Days	G.M. Conc. $CoCl_2$	Growth After 3 Days	G.M. Conc. $CoCl_2$	Growth After 3 Days	G.M. Conc. $CoCl_2$	Growth After 3 Days	G.M. Conc. $CoCl_2$	Growth After 3 Days
.00050[1]	−	.00050	−	.000080	−	.00080	−	.0400	−
.00040	−	.00040	−	.000070	−	.00070	−	.0350	−
.00030[1]	+	.00030	−	.000060	−	.00060	+	.0300	−
.00020	−	.00020	−	.000050	−	.00050	+	.0250	−
.00010	−	.00010	−	.000040	−	.00040	+	.0200	−
.00009	+	.00009	+	.000030	−	.00030	+	.0150	−
.00008	+	.00008	+	.000020	+	.00020	+	.0100	+
.00007	+	.00007	+	.000015	+	.00020	+	.0050	+
.00006	+	.00006	+	.000010	+	.00009	+	.0010	+
.00005	+	.00005	+	.000008	+	.00008	+	.0008	+

The influence of the different media on cobaltous chloride is quite marked. It is five hundred times more toxic in lactose than in peptone. In starch it is less toxic than in glucose or saccharose but more toxic than in peptone. The average limit concentration is .0020 molar, which shows that, on the whole, it is only a little more toxic than zinc chloride. The poisoning of the fungus by cobalt, cadmium and mercury is different from that of calcium, iron, etc., in that *Monilia* in nature probably does not come in contact with any but minimal concentrations of the salts of these metals. Its resistance to cobaltous chloride, cadmium chloride

[1] The growth in this culture was quite limited; only a small per cent. of the spores had pushed out germ tubes.

and mercuric chloride is, therefore, not due to any acquired relation of immunity or susceptibility.

TABLE X

THE TOXICITY OF CADMIUM CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $CdCl_2$	Growth After 3 Days	G.M. Conc. $CdCl_2$	Growth After 3 Days	G.M. Conc. $CdCl_2$	Growth After 3 Days	G.M. Conc. $CdCl_2$	Growth After 3 Days	G.M. Conc. $CdCl_2$	Growth After 3 Days
.00050	−	.00050	−	.00050	−	.00090	−	.0150	−
.00030	−	.00040	−	.00040	−	.00080	−	.0100	−
.00010	−	.00030	−	.00030	−	.00070	−	.0050	+
.00008	−	.00020	+	.00020	−	.00060	−	.0040	+
.00006	+	.00010	+	.00010	+	.00050	+	.0030	+
.00004	+	.00008	+	.00008	+	.00040	+	.0020	+
.00002	+	.00006	+	.00006	+	.00030	+	.0010	+
.00001	+	.00004	+	.00004	+	.00020	+	.0008	+
.000008	+	.00002	+	.00002	+	.00010	+	.0006	+
.000006	+	.00001	+	.00001	+	.00008	+	.0004	+

The toxicity of cadmium chloride is shown in Table X. It is most toxic in saccharose and least toxic in peptone. In glucose it is more toxic than in starch, but less toxic than in lactose. It is more than three times as toxic in saccharose as in glucose. Its average limit concentration in the different media is .001 molar. In saccharose, starch and peptone, cadmium chloride is more toxic than cobaltous chloride. In glucose and lactose, on the other hand, cobaltous chloride is more toxic than cadmium chloride.

TABLE XI

THE TOXICITY OF MERCURIC CHLORIDE IN SACCHAROSE, GLUCOSE, LACTOSE, STARCH, AND PEPTONE MEDIA

Saccharose 5%		Glucose 5%		Lactose 5%		Starch 5%		Peptone 5%	
G.M. Conc. $HgCl_2$	Growth After 3 Days	G.M. Conc. $HgCl_2$	Growth After 3 Days	G.M. Conc. $HgCl_2$	Growth After 3 Days	G.M. Conc. $HgCl_2$	Growth After 3 Days	G.M. Conc. $HgCl_2$	Growth After 3 Days
.000400	−	.00050	−	.000200	−	.000050	−	.0055	−
.000200	−	.00040	−	.000100	−	.000040	−	.0050	−
.000100	−	.00030	−	.000080	−	.000030	−	.0045	−
.000080	−	.00020	−	.000060	−	.000020	−	.0040	−
.000060	+	.00010	+	.000040	−	.000010	+	.0035	−
.000040	+	.00008	+	.000020	+	.000008	+	.0030	−
.000020	+	.00006	+	.000010	+	.000006	+	.0025	−
.000010	+	.00004	+	.000008	+	.000004	+	.0020	+
.000005	+	.00002	+	.000006	+	.000002	+	.0015	+
.000001	+	.00001	+	.000004	+	.000001	+	.0010	+

Overton (22) has pointed out that mercuric chloride, which differs from most salts in being more soluble in ether, lanolin, etc., exerts its poisonous action more quickly than the other salts of the heavy metals and thinks that this fact favors the view that the plasma membrane is a lipoid layer about the cell. The data in Table XI supports the evidence for the great toxicity of this salt in all of the different media used. It is, however, more toxic in starch and less toxic in peptone than in any of the other media. It is two hundred times more toxic in starch than in peptone. It is also more toxic in lactose than in glucose or saccharose. It is, as noted, the most toxic of all the chlorides used, its average limit concentration being .0004 molar. A comparison of the limit concentrations of the chlorides of zinc, cadmium and mercury shows that the toxicity of these salts is roughly proportional to the atomic weights of zinc, cadmium and mercury. Mercury is somewhat more toxic than would be expected from its atomic weight but the relation shown here suggests that a better knowledge of the toxicity of non-nutrient salts in different media may further support the view that toxicity is related to the periodic functions of the atomic weights of the elements.

TABLE XII

THE TOXICITY OF VARIOUS CHLORIDES IN FIVE DIFFERENT MEDIA

	KCl	NH_4-Cl	Na-Cl	Ca-Cl_2	Ba-Cl_2	$FeCl_3$	$CuCl_2$	$ZnCl_2$	$CoCl_2$	$CdCl_2$	$HgCl_2$
Saccharose . .	1.8	.7	.26	.50	.30	.00010	.00090	.00040	.00009	.00060	.00006
Glucose.	1.7	1.0	.80	.70	.20	.00010	.00080	.00010	.00009	.00020	.00010
Lactose.	1.1	.6	.50	.12	.35	.00001	.00003	.00070	.00002	.00010	.00002
Starch.	.8	.7	.80	.50	.20	.00080	.00045	.00006	.00060	.00050	.00001
Peptone.	1.1	.7	.75	.40	.25	.03500	.02000	.01000	.01000	.00500	.00200

Table XII gives the limit concentration of each salt in each medium used. A comparison of these values brings out some interesting relations. Taking the average concentrations of the different chlorides in the various media, we see that the alkali and alkali earth salts which were tested are most toxic in saccharose and least toxic in glucose. The heavy metals are most toxic in lactose and least toxic in peptone. While potassium chloride is most toxic in starch and least toxic in saccharose, sodium chloride is least toxic in starch and glucose and most toxic in saccharose.

Ammonium chloride and calcium chloride are most toxic in lactose and least toxic in glucose. Barium chloride, however, is least toxic in lactose and most toxic in glucose and starch. Cupric chloride, ferric chloride and cobaltous chloride are most toxic in lactose. Zinc chloride and mercuric chloride are most toxic in starch. Cadmium chloride is most toxic in saccharose. None of the chlorides of the alkali or alkali earth metals, but all of the chlorides of the heavy metals are least toxic in peptone media. In lactose media, ferric chloride is more toxic than any of the other chlorides; in glucose media, cobaltous chloride is most toxic; in starch, peptone and saccharose media, mercuric chloride and cadmium chloride are most toxic. Potassium chloride in all of the media used is the least toxic of all of the salts tested, except sodium chloride in a starch medium. Potassium chloride in saccharose is less toxic, and mercuric chloride in starch is more toxic than any of the other salts in any of the other media. Reading from right to left in Table XII, one sees the increasing toxicity of the different salts. The arrangement of the salts in the order of their toxicity is different for each of the five different kinds of media.

In order to obtain evidence relative to the number of free ions in the various media containing limit concentrations of the same salt, a number of tests were made of the electrical resistances of the different media. The results of some of these tests are given in Table XIII.

TABLE XIII

THE ELECTRICAL RESISTANCE OF SOME OF THE MEDIA

Medium	G.M. Conc. of $FeCl_3$	Resistance in Ohms	G.M. Conc. of $CuCl_2$	Resistance in Ohms	G.M. Conc. of $CdCl_2$	Resistance in Ohms	G.M. Conc. of $HgCl_2$	Resistance in Ohms
Saccharose....	.00010	3,700	.00090	8,900	.00006	6,500	.00006	14,400
Glucose.....	.00010	4,900	.00080	10,100	.00020	5,000	.00010	6,900
Lactose.....	.00001	5,600	.00003	——	.00010	7,100	.00002	12,500
Starch.....	.00080	4,200	.00045	1,600	.00050	1,200		
Peptone.....	.03500	10	.02000	97	.00500	160	.00200	200

The tests of electrical resistance were made by means of the Wheatstone bridge method, a Freas electrolytic cell being used. The resistances shown by five per cent. solutions of the organic substances used have already been given. Table XIII shows in a

striking way the low resistance of peptone media and indicates that *Monilia* is actually able to endure higher concentrations of these poisonous substances when it is growing in peptone media than when it is in any of the other media which have been used. The table also suggests the probability that the susceptibility of the fungus to the same kind of ions varies according to the medium in which it is growing.

In an attempt to further elucidate the nature of the toxic action of the various salts on *Monilia*, I have also attacked the problem from the standpoint of the hypothesis that failure of the spores to germinate in a medium containing less than the lethal dose of a toxic substance is due to the inability of the protoplast to absorb sufficient water. Careful observation of the appearance and behavior of spores in toxic media has led to the belief that the retarding action of a toxic salt on germination and growth is the result of its influence on the ability of the cells to take up water. As has already been stated, the highest concentration of a salt in which *Monilia* spores show germination after three days, has been designated the limit concentration for that medium. It is recognized that this is an arbitrary standard. The limit concentrations given in the above tables hold only in the case of an incubation period of three days. Germination will take place in concentrations which are greater than this limit concentration, provided the spores are left in the medium for a longer period of time.

Spores placed in a medium in which the toxic substance is slightly more dilute than the limit concentration, germinate and produce mycelia. The rate of growth in such a medium is much slower than in a non-toxic medium. The most obvious characteristic of a poisoned culture is the long incubation period and the slow rate of growth. The effect of potassium chloride on the time of germination of spores of *Monilia* on a potato medium (thirty-five per cent. potato cubes) is shown by Table XIV.

While growth becomes visible in twenty-one hours in a medium containing potassium chloride at a concentration of .62 molar, it takes thirty-four hours when the concentration is 1.26 molar and fifty-five hours when the concentration is 1.9 molar. This experiment illustrates the behavior of the fungus in media which are toxic but which do not entirely inhibit growth.

TABLE XIV

THE EFFECT OF POTASSIUM CHLORIDE ON RATE OF GROWTH

Concentrations of KCl	Incubation Period	Concentrations of KCl	Incubation Period
0.62 molar.	21 hours.	1.42 molar.	36 hours.
0.70 "	22 "	1.50 "	37 "
0.78 "	23 "	1.58 "	38 "
0.86 "	25 "	1.66 "	40 "
0.94 "	27 "	1.74 "	42 "
1.02 "	29 "	1.82 "	44 "
1.10 "	31 "	1.90 "	55 "
1.18 "	32.5 "	1.98 "	59 "
1.26 "	34 "	2.06 "	63 "
1.34 "	35 "	2.14 "	No germination after 20 days.

If now we study the condition of the spores in media that entirely inhibit germination, we obtain some interesting data. The spores will remain viable for two weeks or longer in the presence of so toxic a substance as mercuric chloride, provided the concentration is slightly below that which causes plasmolysis. Some spores which had been kept for two weeks on a starch medium containing mercuric chloride at a concentration of .00005 molar germinated when transferred to potato agar. This shows that although the spores do not germinate in such a toxic medium, they are not much injured by it. The toxic substances which I have used cause serious injury to the spores, only when they are concentrated enough to bring about plasmolysis. At concentrations less than this, the spores do not germinate but they remain alive for a long time. At still lower concentrations they not only remain alive but germinate and make a slow growth.

This slow growth in media containing approximately limit concentrations of toxic substances seems to me strong evidence that although the poison is not present in sufficient quantities to cause plasmolysis, it nevertheless hinders the absorption of water by the spores and in this way inhibits their growth. This assumption seems fully in accord with the facts above noted. As the concentration of the toxic substance is increased, the ability of the protoplasm to absorb water becomes less and less and the time required for germination longer and longer. A concentration is finally reached at which the spores are no longer able to absorb any water from the surrounding medium. Although this con-

centration inhibits germination, it does not kill the spores, even after considerable periods of time. When the concentration is still further increased the water holding power of the spores becomes less and plasmolysis results.

If as is here assumed the rate of growth of the fungus in a toxic medium depends on its rate of water absorption, then any means by which the water content of the mycelium could be lessened should also decrease the rate of growth. If, for example, *Monilia* be placed in a dry atmosphere where the loss of water by the aerial part of the mycelium would be great, the rate of growth should be correspondingly decreased. The following experiments show the effect of a dry atmosphere on the rate of growth of the fungus mycelium.

The media used in this work consisted of cubes of potato to which was added enough water to fill the bottom of the culture vessel to depth of about one half of a centimeter. On such a substratum *Monilia* makes very abundant growth. The cultures were incubated at a temperature of 29° C. This is one degree below the temperature most favorable for its growth (see Went, 34). Since the effect of drying the atmosphere over cultures of *Monilia* varies somewhat with the age of the culture at the time the drying agent is used, a few remarks regarding the appearance of the fungus at different stages in its development seem desirable at this point.

Under the conditions outlined above, growth first becomes visible to the naked eye after an incubation period of from nine to ten hours. During the next fifteen hours the mycelium grows so rapidly that it almost hides the surface of the potato cubes. In the next seven hours the fungus makes still more rapid growth, rising from five to ten centimeters above the culture medium. This is followed by a period of about six hours, during which there is little visible change. When the cultures are approximately forty hours old they begin to take on a beautiful pink color and during the next hour spore formation begins.

If the vapor pressure above cultures that are more than thirty hours old is unduly lowered, the mycelium withers and fails to produce spores. An entirely different result is obtained in the case of young cultures. If five cubic centimeters of a four molar

potassium chloride solution be suspended in an oiled paper bag above a young culture, it checks the growth of the mycelium to a remarkable extent. The less concentrated the solution in the paper bag, the greater will be the mycelial growth in the culture above which it is suspended. Drying the air by placing small amounts of calcium chloride over the cultures gives still more striking results. The mycelium can be kept from rising more than a few millimeters above the surface of the medium. Table XV shows the effect of suspending potassium chloride solutions of different concentrations over cultures kept under conditions that were otherwise identical. The bags containing the solutions were placed in the bottles at the time the inoculations were made.

TABLE XV

INFLUENCE OF HUMIDITY ON THE GROWTH OF *Monilia*

Solution Suspended Above Culture	Height of Mycelium After 32 Hours
5 c.c. of 4.0 molar KCl solution.	1.0 cm.
5 c.c. " 3.2 " KCl "	1.5 "
5 c.c. " 2.4 " KCl "	3.0 "
5 c.c. " 1.6 " KCl "	3.5 "
5 c.c. " .8 " KCl "	3.2 "
5 c.c. " distilled water	4.0 "

FIG. 1 shows the six cultures referred to in Table XV. The photograph was taken several hours after obtaining the measurements used in the table. The culture that made the least growth is the one above which was suspended five cubic centimeters of four molar potassium chloride solution. Distilled water was suspended over the culture that made the greatest amount of growth. As the rate of loss of water by the mycelium is increased the rate of growth is correspondingly decreased. By suspending one gram of anhydrous calcium chloride over alternate cultures (FIG. 2) the influence of vapor pressure on rate of growth is still more strikingly shown. The amount of water taken up by the calcium chloride, however, is very small.

In order to determine the amount of water taken up by calcium chloride suspended over cultures of *Monilia* when it causes such variations in growth as are to be noted in FIG. 2, bags were weighed before and after being suspended over cultures. The difference in weight gives the amount of water taken up by the

drying agent. This amount was found to be in ten instances respectively: 1.32g; 1.45g; 1.40g; 1.32g; 1.38g; 1.43g; 1.39g; 1.36g; 1.30g; 1.37g or an average of 1.33 grams. A similar calcium

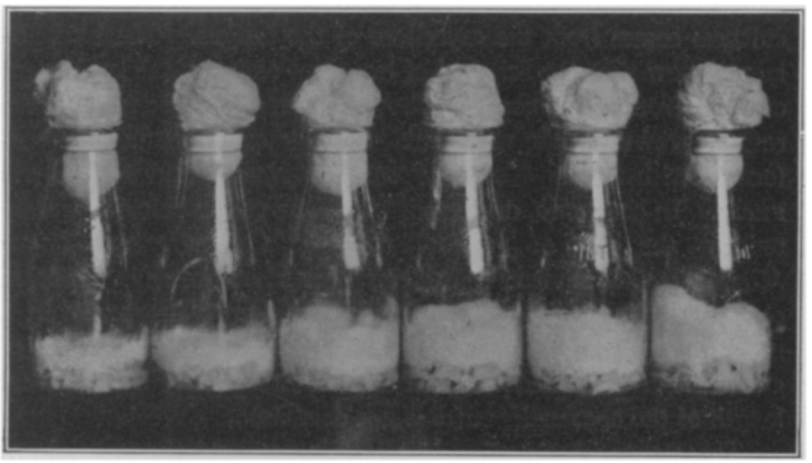

FIG. 1. Cultures of *Monilia sitophila*. This photograph shows the influence of humidity of the atmosphere on the rate of growth of *Monilia*. An oiled paper bag, containing a 4 molar potassium chloride solution, was suspended over the culture that shows the least growth, while distilled water was suspended over the culture that made the greatest growth. The series of six cultures shows the effect of drying the air over the mycelium to different degrees.

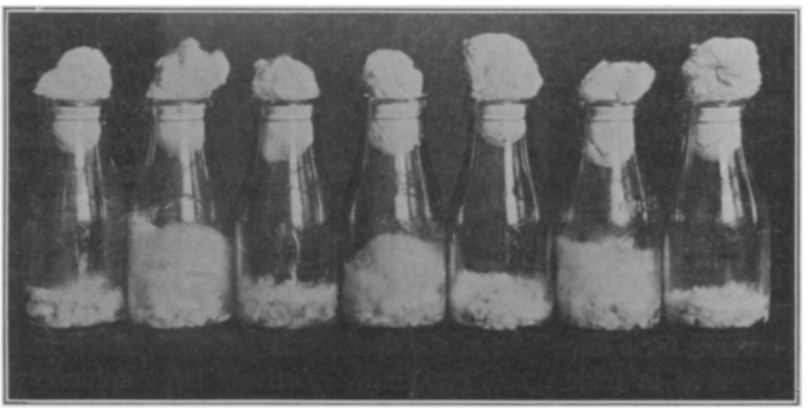

FIG. 2. Cultures of *Monilia sitophila*. This photograph shows the influence of drying the air over young cultures of *Monilia*. One gram of anhydrous calcium chloride was suspended above the cultures in alternate bottles; the small amount of growth made by the fungus in these bottles is shown.

chloride bag was suspended for the same length of time over a sterile medium exactly like that on which the *Monilia* was being grown. This bag took up only .39 gram of water, showing that most of the water taken up by the drying agent suspended over cultures comes from the mycelium of the fungus. These experiments show that the removal of less than one and one half grams of water from the atmosphere above cultures greatly decreases the rate of growth of the mycelium. Similar experiments were performed with *Mucor Mucedo*, *Sporodinia grandis* and *Phycomyces nitens*. In each case, drying the air above young cultures greatly reduces the rate of growth. *Sporodinia grandis* and *Mucor Mucedo* produce shorter sporangiophores when grown in a culture over which a drying agent is suspended than when grown in a moist atmosphere. In the case of *Phycomyces nitens* the rate of growth is greatly checked by drying the atmosphere but the final length of the sporangiophores is not decreased.

These experiments show in a striking manner the well known importance of water to growth. They also suggest that the slow germination and the slow rate of growth in toxic solutions of potassium chloride may be largely due to the lowering of the vapor pressure above the medium. The same thing is probably true of all other salts that must be used in considerable concentrations in order to inhibit growth. But it is quite different with more toxic substances such as the salts of the heavy metals which inhibit growth when present in very small quantities. Their effect on the vapor tension of the medium is, of course, insignificant. This difference between toxic and relatively non-toxic substances has not been sufficiently emphasized. It seems to me that the above experiments offer strong evidence in support of the assumption that an important factor in many cases of toxicity is the effect of the poison on the ability of the protoplast to absorb water. They, at least, show that this factor is sufficient to account for a large part of the decrease in growth which is to be observed in toxic media containing relatively large amounts of dissolved salts.

A salt like potassium chloride when added to a medium in sufficient quantities to check growth, increases the affinity of the medium for moisture and in this way makes it difficult for the

mycelium to take up sufficient water. By lowering the vapor tension of the medium it also increases the rate at which water is lost by the fungus. If instead of adding the salt to the medium it is dissolved in a small amount of water and suspended above a young culture the strong solution takes up water from the air. In this case the salt does not retard the rate at which water is taken up by the mycelium but does increase the rate at which water is given off. It is very interesting to see that when the salt is suspended above the growing culture it causes a decrease in the rate of growth which is similar to that to be observed when it is added directly to the medium. There can be little doubt that in media containing toxic concentrations of potassium chloride, the retardation in growth is largely the result of the inability of the fungus to absorb and to retain sufficient water. Though toxic concentrations of the salts of the heavy metals do not appreciably lower the vapor pressure above the medium, it is possible that these salts also act by decreasing the power of the cells to absorb water.

A consideration of the factors which may be involved in bringing about the variations in toxicity in the different media leads to the suggestion of several possibilities. In the first place it is known that certain salts react with sugars, starch and proteids. The resulting compounds if formed in a given medium would be expected to show properties different from the salt and different from other compounds that might be formed in still other media. Relatively little is known of these substances, but some of them have been studied and the conditions that favor their formation have been determined.

In a solution of sodium hydroxide, cane sugar is converted into sodium saccharate (see Thomsen, **32**). Saccharates of calcium, potassium and barium are also known (see Peligot, **24**). All of these saccharates are formed by the action of the hydroxides of the different metals on cane sugar. The addition of ammonia to a solution of cane sugar increases the rotatory power of the solution and has been taken as evidence that a compound is formed between the sugar and ammonia (see Wilcox, **35**). Copper and iron saccharates have also been reported (see Graham, **10**). They are made by adding the chlorides to alkaline solutions of cane sugar. We can not, however, in the cases before us, attribute the variations in

toxicity of different salts in cane sugar media to the production of saccharates, since the addition of chlorides to a neutral solution of cane sugar does not furnish the conditions necessary for their formation. In order to obtain saccharates it would be necessary to add alkalies to these solutions. There seems to be good evidence, however, that some of the chlorides do form loose combinations with cane sugar (see Peligot, **24**). A compound represented by the formula $2C_{12}H_{22}O_{11}.BaCl_2$ has been prepared in crystalline form (Gauthier, **8**). It may be that such addition compounds are factors in determining the relative toxicity of the chlorides in saccharose media. Barium chloride is less toxic in saccharose than in starch, glucose or peptone. It may be that this rather low toxicity in saccharose is due to the formation of the compound referred to above.

Compounds of glucose and lactose, analogous to the saccharates, are also known (Hönig & Rosenfeld, **11**, **12**). They are formed by the action of alkalies on these sugars. With glucose, sodium chloride and potassium chloride form compounds which are represented by the formulae $C_6H_{12}O_6.NaCl$ and $C_6H_{22}O_6.KCl$ respectively (Gladstone, **9**). It may be that the rather low toxicity of sodium chloride and potassium chloride in glucose media is due to the formation of these compounds. Starch has been shown to have the properties of a very weak acid and to be able to react with small quantities of neutral salts (see Demoussy, **5**). Sodium chloride is less toxic in starch than in any of the other media except glucose. It may be that some of the salt has combined with starch to give a compound that is less toxic than sodium chloride. Potassium chloride, on the other hand, is more toxic in starch than in any of the other media. It is possible that this is due to the formation of a complex compound which is more toxic than the chloride.

The addition of any of the chlorides to a five per cent. peptone solution always causes a certain amount of precipitation. That the salts are to some extent carried down by this precipitate seems highly probable. Pauli (**23**) has shown that neutral proteids adsorb electrolytes. The antitoxic action of peptone on the salts of the heavy metals may be in part due to this adsorption. Table XII shows that the toxicity of all of the chlorides of the heavy metals is less in peptone than in any of the other media.

Although some of the variations in the toxicity of the chlorides in the different media may be the result of reactions between the salts and organic substances, there is good evidence that this can not account for all of the variations brought out in Table XII. A study of some of the media from the standpoint of their resistance to the passage of the electric current has shown that there is considerable variation in the ionic concentration of different media that contain limit concentrations of the same salt, indicating that some media do influence the concentration of the ions but that these differences are not correlated directly with the observed differences in toxicity. Limit concentrations of ferric chloride, cupric chloride, cadmium chloride and mercuric chloride in peptone show in each case a much lower resistance than limit concentrations of these same salts in saccharose, glucose, lactose or starch. This seems to be strong evidence in support of the view that the fungus is actually able to resist higher ionic concentrations of these salts when it is growing in peptone than when it is in any of the other media tested, thus showing an effect of the peptone other than that due to its precipitation of a portion of the toxic salt.

Another factor of probable importance in this connection is the influence of the different media on the production of enzymes. Went (34) has shown that *Monilia sitophila* produces a number of different enzymes and that the organic part of the medium determines which enzyme will be produced in any given case. The effect of the various chlorides on trypsin, which the fungus produces in peptone media, may be quite different from their influence on diastase produced in starch media. The rather high toxicity of potassium chloride and barium chloride in starch media may be due to the infleunce of these salts on diastase. Similar variations may be brought about by the actions of the poisons on lactase, lipase and invertase.

My observations show clearly that the organic part of the medium must be taken into account in studies on toxicity. This important factor, however, has generally been disregarded by those who have tested the resistance of fungi and bacteria to poisons. Stevens (30) tested the toxic action of mercuric chloride and other poisons on *Penicillium crustaceum* growing in bread media. In a study of the toxicity of a whole series of substances for *Aspergillus*

flavus, *Sterigmatocystis nigra*, *Oedocephalum albidum*, *Penicillium glaucum* and *Botrytis vulgaris*, Clark (4) used a sugar beet infusion. Klebs (14) determined the poisonous influence of various substances on *Saprolegnia mixta* growing in a pea infusion. Bessey (2) tested the resistance of fungi to copper sulphate, mercuric chloride and other poisons without taking into account the possible influence of organic materials in the synthetic media which he used.

Pulst (25) measured the toxicity of copper sulphate, zinc sulphate and nickel sulphate. He tested the action of these salts on *Mucor Mucedo*, *Aspergillus niger*, *Botrytis cinerea* and *Penicillium glaucum* in a medium containing sugar and peptone, without regard to the effect of sugar or peptone on the toxicity of the salts.

Loew (20) has studied the poisonous action of sodium fluoride on *Bacillus mycoides*, *B. pyocyaneus*, *B. subtilis* and *B. prodigiosus*. Using a bouillon medium, he found that these bacteria could endure approximately one per cent. of sodium fluoride. He, therefore, disagrees with Arthus and Huber (1) who held that a one per cent. solution of this salt is deadly to all cells. He also compares the toxic action of sodium fluoride on *Spirogyra communis* in an aqueous solution with its effect on bacteria in a bouillon medium and notes that the bacteria have a much higher resistance than the alga.

Ssadikow (29) studied the resistance of *Bacillus subtilis* to strychnin salts in bouillon, nutrient agar and nutrient gelatin. My observations suggest that the high concentrations which this organism was able to endure might have been fatal to it in a medium containing no peptone. Renard (27) has tested the antitoxic action of different concentrations of each of twelve nutrient salts on the poisonous effects of eleven different toxic substances, mostly chlorides and nitrates of the heavy metals. He finds that in general, the antitoxic action increases with the concentration of the inhibiting salt. Except in a few cases where the antagonistic substance is a salt of an organic acid, he has made his tests in glucose media. The antitoxic coefficients by which he attempts to express the relations between the several poisons and their antidotes would undoubtedly have been found to be different for each medium tested if he had tried other common organic sub-

stances. He has not taken into account the influence of the glucose on the toxicity of the poisons used although in an experiment in which he studies the action of copper salts in the presence of potassium acetate both with and without glucose, he obtains evidence that the glucose lessens the toxicity of the copper. Although Lipman (**17**) makes mention of the fact that sodium carbonate is much less toxic to ammonifying organisms in certain soils than in pure peptone solutions, he has, nevertheless, drawn conclusions as to the toxicity of various salts after testing them in a medium consisting of soil and dried blood, without taking into account the effect of the organic substances on the salts which the soil must have contained.

My results show that toxicity measurements which are made without regard to the organic substances in the medium are of little value as indicating the relative resistance of different organisms. The bearing of the facts here brought out on a number of practical problems is evident. They must be reckoned with in considering the influence of the decomposition products of humus in the soil, on soil toxins, in the preparation of antidotes, in the chemical sterilization of water and in the preservation of milk and other food materials by chemical means.

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